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Research Note

Evaluation of Various Antimicrobial Interventions for the Reduction of *Escherichia coli* O157:H7 on Bovine Heads during Processing†

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ABSTRACT

The effectiveness of electrolyzed oxidizing water, FreshFx, hot water, DL-lactic acid, and ozonated water was determined using a model carcass spray-washing cabinet. A total of 140 beef heads obtained from a commercial processing line were inoculated with *Escherichia coli* O157:H7 on the cheek areas. Each head was exposed to a simulated preevisceration wash and then had antimicrobial wash treatments. Hot water, lactic acid, and FreshFx treatments reduced *E.* coli O157:H7 on inoculated beef heads by 1.72, 1.52, and 1.06 log CFU/cm^2, respectively, relative to the simulated preevisceration wash. Electrolyzed oxidizing water and ozonated water reduced *E. coli* O157:H7 less than 0.50 log CFU/cm^2. Hot water, lactic acid, and FreshFx could be used as decontamination washes for the reduction of *E. coli* O157:H7 on bovine head and cheek meat.

Variety meats, including head and cheek meats, have been determined to carry a higher level of microorganisms than does other meat animal tissue, either by nature and origin or by poor hygienic and chilling conditions *(10, 12).* Contamination of bovine heads also occurs during carcass washing after hide removal as contaminants are washed down the carcass. Carney et al. *(3)* reported prevalence of *Escherichia coli* O157:H7 found on head meat to be 3% and at levels ranging from 0.7 to 1.0 log CFU/g. Typically, head meat and cheek meat are removed from the carcass prior to the application of the final antimicrobial interventions. Reducing the bacterial contamination on beef carcasses has been of primary concern for several years. The use of the same techniques, however, is not as well established in the processing of variety meats. Because beef cheek meat can be used in the preparation of ground beef, chopped beef, or fabricated beef steaks *(23),* any cheek meat harboring *E. coli* O157:H7 may become another source of contamination of ground beef. The objective of this study was to evaluate the effectiveness of various compounds either currently in use by the beef industry or commercially available for use in a beef head wash cabinet to reduce *E. coli* O157:H7 on cheek meat.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and inoculum preparation. *E. coli* O157:H7 strain ATCC 43888, lacking both Shiga toxins, was used in this study. The phenotype and growth characteristics of this strain are similar to those of toxigenic strains *(1).* This strain is also reported to have a similar susceptibility to antimicrobial compounds compared with *E. coli* O157:H7 isolated from human and generic *E. coli (21).* The cells were grown in tryptic soy broth (TSB) without glucose and supplemented with 0.6% yeast extract (Difco, Becton Dickinson, Sparks, Md.) for 16 to 18 h at 37°C. The cells were diluted 100-fold with 10× nutrient broth (Difco, Becton Dickinson) for use in the viability loss and injury studies. For bovine head intervention treatments, the cells were diluted 10-fold with sterile 0.1% peptone solution. The cells were chilled in an ice bath before use to slow down the growth rate.

Determination of pathogen reduction and sublethal injury. One hundred microliters of *E. coli* O157:H7 in 10× nutrient broth were inoculated into 900 μl of acidic electrolyzed oxidizing water (EO; pH 2.8, 60 ppm chlorine with 1,190 mV of oxidation-reduction potential; Electric Aquagenics, Kennesaw, Ga.), FreshFx (1:50, pH 1.6; SteriFX, Inc., Shreveport, La.), DL-lactic acid (2%; Sigma, St. Louis, Mo.), and ozonated water (OZ; ≥2.3 ppm and 25°C; Ozone International, Bainbridge Island, Wash.) in a 96-deep-well block and mixed by pipetting up and down five times with a 12-chanel pipettor. The final pH values of treated medium ranged between 1.7 and 3.1. The deep-well block was incubated at room temperature for either 12 or 26 s. The 12-s exposure time was based on the chain speed at the processing plant (300 heads per h), while the 26 s was based on the head chain speed after removal from the carcass. For hot water treatment, 300 μl of *E. coli* O157:H7 in 10× nutrient broth was inoculated into 2,700 μl of deionized water in a test tube (16 by 100 mm) that was tempered at 74°C in a water bath. The cells were mixed and incubated for either 12 or 26 s before cooling with tap water. After each time interval, both controls and treated samples were serially 10-fold diluted with TSB (Difco, Becton

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Dickinson) supplemented with 0.017 M KH_2PO_4 and 0.072 M K_2HPO_4 (TSB-PO_4; Sigma), pH 7.2 ± 0.1, to neutralize acid residue. An appropriate dilution was spiral plated (Spiral Biotech, Norwood, Mass.) on both nonselective tryptic soy agar (Difco, Becton Dickinson) with 0.6% yeast extract to improve growth of stressed cells, and on selective media CHROMAgar O157 (DRG International, Mountainside, N.J.) supplemented with 5 mg of novobiocin per liter and 1 mg of potassium tellurite per liter (nt-CHROMAgar) in duplicate. Plates were incubated at 37°C for 16 to 18 h. The injured cells were determined from the differences of these two media *(19).* The experiment was repeated three times.

Preparation of bovine heads. A total of 140 bovine heads with hide removed were collected after U.S. Department of Agriculture inspection from a commercially fed beef processing plant. Bovine heads were held in a room at 29°C, and the cheek areas were reassembled to their original positions with the aid of deadlock pins and disposable tissue staplers (3M Health Care, St. Paul, Minn.). Before inoculation (approximately 5 to 7 min of assembling time), both sides of the cheek area were marked with edible ink, using sterile cotton swabs and a 100-cm^2 template and divided into equal quadrants of 25 cm^2. Each side of each head served as an observation for each intervention.

Inoculation, intervention treatments, and sampling of bovine heads. Three hundred microliters of *E. coli* O157:H7 in 0.1% peptone solution was applied onto the surface of the marked areas and evenly spread with the back of sterile plastic spoon. A 15-min waiting period before subjecting to interventions was provided to allow for cell attachment on the tissue to occur. The initial bacterial load was approximately 1×10^6 CFU/cm^2.

Intervention treatments were conducted in the top half of a commercial beef carcass wash cabinet (Chad Co., Olathe, Kans.) mounted into a polypropylene cabinet (4.3 by 1.2 by 1.6 m) modified for beef head spray washing. A series of nozzles was oscillated at the speed of 80 cycles per min. Before intervention treatments, bovine heads were spray washed inside the cabinet with water (25 ± 2°C) for 10 s at 45 lb/in^2, followed by water (74 ± 2°C) for 10 s at 10 lb/in^2 to simulate a preevisceration carcass wash. The distance between the head and the nozzles was 20 cm. All antimicrobial compounds were sprayed at the rate of 14 liters/min. However, the rate of water and hot water sprayed on beef heads was not determined. Hot water was applied for 26 s at 10 lb/in^2 and at 74 ± 2°C. Both lactic acid and FreshFx solutions were sprayed for 26 s at 25 lb/in^2 and at 25 ± 2°C. Two different studies to evaluate the efficacy of EO and OZ were conducted. Generally, the production of EO yields two different types of EO water, acidic EO and alkaline EO *(15).* The acidic EO (EO-I) was sprayed as a final rinse for 26 s at 25 lb/in^2 and 25 ± 2°C. To keep the same treatment time of 26 s, the alkaline EO (EO-II) water was applied to bovine heads for 13 s at 25 lb/in^2, followed by EO-I for 13 s at 25 lb/in^2 as a final rinse. Similarly, two different treatments for OZ also were evaluated for efficacy in reducing *E. coli* O157:H7. For the first treatment (OZ-I), OZ was applied as a final rinse for 26 s at 25 lb/in^2 and 25 ± 2°C. For the second treatment (OZ-II), a high-pressure water wash (HP; 145 lb/in^2 at 25°C) was applied for 6 s, followed by OZ for 20 s at 25 lb/in^2 as a final treatment. Ozone and free chlorine concentrations were determined with an ozone test kit (Hach, Loveland, Colo.) and chlorine test strips (Industrial Test Systems, Rock Hill, S.C.), respectively. The pH of EO was determined using a portable pH meter (Accumet, Fisher Scientific, Pittsburgh, Pa.).

Three surface samples of cheek meat were randomly and aseptically excised from three of the quadrants of the marked area. One quadrant each was sampled before entering spray wash cabinet (control), after prewash, and after applying the intervention. The tissue was then placed into sterile, filtered Whirl-Pak bags (Nasco, Ft. Atkinson, Wis.) and held at room temperature for approximately 10 min to simulate the time from head wash to fabrication room. The total time between inoculation and microbiological analysis was approximately 30 min.

TABLE 1. *Inactivation and sublethal injury of* E. coli *O157:H7 caused by various antimicrobial compounds*

Treatment group[a]	*n*	Time (s)	Log CFU/ml[b]	Log reduction[c]	Log injured cells[d]
Control	6		5.96 A	—	—
EO	9	12	5.41 B	0.55	0.92 A
EO	9	26	5.20 C	0.76	2.33 B
Control	6		6 A	—	—
FreshFx	9	12	5.18 B	0.82	1.77 A
FreshFx	9	26	5.01 C	0.99	4.05 B
Control	6		6.15 A	—	—
Hot water	9	12	3.16 B	2.99	2.05 A
Hot water	9	26	2.6 C	3.55	2.15 A
Control	6		6.05 A	—	—
Lactic acid	9	12	4.62 B	1.43	2.2 A
Lactic acid	9	26	3.99 C	2.06	3.73 B
Control	6		6.09 A	—	—
OZ	9	12	5.84 B	0.25	1.11 A
OZ	9	26	6.16 AC	−0.07	1.52 B

[a] EO, acidic electrolyzed oxidizing water; OZ, ozonated water.
[b] Means within the same treatment group bearing the same letter do not differ significantly at $P \leq 0.05$.
[c] Reduction was determined from the difference of bacterial population before and after treatment on nonselective medium.
[d] Injured cells were determined from the difference of bacterial population after treatment on nonselective and selective media. Means within the same treatment group bearing the same letter do not differ significantly at $P \leq 0.05$.

Microbiological analysis of inoculated bovine heads. A 50-ml aliquot of TSB-PO_4 was aseptically added into each tissue sample bag. Each bag was agitated vigorously (540 rpm) for 1 min, using a stomacher (BagMixer 400, InterScience, Weymouth, Mass). The contents in the bag were 10-fold serially diluted with buffered peptone water (Becton Dickinson), and appropriate dilutions were spiral plated on ntCHROMAgar in duplicate. The plates were incubated at 37°C for 16 to 18 h, and presumptive colonies were tested for the O157 antigen, using an agglutination test kit (DrySpot *E. coli* O157, Oxoid, Basingstoke, UK). Three presumptive colonies from each treatment were further confirmed to be the inoculated strain by multiplex PCR reactions *(13).* Data were analyzed by analysis of variance using a completely randomized design (SAS Institute, Inc., Cary, N.C.). Least-squares means were calculated and pairwise comparisons of means were determined using Tukey-Kramer test method with all differences reported at $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Inactivation and sublethal injury of *E. coli* O157:H7. All antimicrobial compounds reduced ($P < 0.05$) *E. coli* O157:H7 in nutrient broth. The magnitude of inactivation of *E. coli* O157:H7 depended on the antimicrobial compounds and the length of exposure time (Table 1). Hot water was the most effective, whereas OZ was the least

effective in reducing *E. coli* O157:H7 for either 12 or 26 s (Table 1). The order of inactivation of the antimicrobials tested in nutrient broth is as follows: hot water > lactic acid > FreshFx > EO > OZ. The small reduction of OZ in this study may be due to less ozone available to inactivate bacterial cells. Ozone is relatively unstable in aqueous solution *(22),* especially at pH higher than 5.0 *(14).* In our study, nutrient broth with a pH of 6.8 was used. Amino acids and peptides also react with ozone at neutral or basic pH *(14),* causing less ozone to interact with bacterial cells.

Lactic acid (2%) was the second most effective compound in killing *E. coli* O157:H7. Both EO and FreshFx had intermediate effects on *E. coli* O157:H7 as compared with hot water and lactic acid. In this study, the effectiveness of EO did not agree with previous studies, in which more than 9 log CFU/ml of *E. coli* O157:H7 were killed after 30 s of EO treatment *(15, 16).* The large reduction caused by EO treatment in that study may be due to the analysis being done in a noncomplex medium of 0.1% peptone solution. In our study, nutrient broth—which is rich in amino acids, peptides, and amines—was used to resuspend *E. coli* O157:H7 cells. Oomori et al. *(18)* found that chlorine generated by EO water was transformed to *N*-chlorate compounds by amino acids and proteins, resulting in less available free chlorine to inactivate bacterial cells. All antimicrobial compounds tested also inflicted injury ($P < 0.05$) to *E. coli* O157:H7 (Table 1) and, generally, a higher degree of cell injury was seen when cells were exposed for 26 s. Among the antimicrobial compounds tested, lactic acid and FreshFx caused the most injury to the cells, followed by hot water, EO, and OZ.

Efficacy of hot water, lactic acid, FreshFx, EO, or OZ on reducing *E. coli* O157:H7–inoculated bovine heads. When evaluated in the spray-wash cabinet, preevisceration wash, hot water, lactic acid, and FreshFx reduced ($P < 0.05$) the *E. coli* O157:H7 on the surfaces of cheek meat (Table 2). The preevisceration wash alone reduced numbers of *E. coli* O157:H7 on inoculated cheek meat at least 1.50 log CFU/cm^2 compared with controls. Hot water, lactic acid, and FreshFx treatments additionally reduced *E. coli* O157:H7 relative to the preevisceration wash. Although FreshFx effects on *E. coli* O157:H7 were similar to those imparted by treatment with lactic acid, reductions in counts of *E. coli* O157:H7 due to treatment with either FreshFx or lactic acid were less than those imparted by treatment with hot water. Several researchers have described the efficacy (1- to 3-log reductions) of hot water and lactic acid for decontamination of carcasses and variety meats *(4, 6–9, 11, 17).* Bosilevac et al. *(2)* reported that hot water was more effective than 2% lactic acid in reducing the prevalence of *E. coli* O157:H7 on preevisceration beef carcasses.

EO-I water or alkaline EO water and then a final rinsing with EO-I water reduced ($P < 0.05$) *E. coli* O157:H7 compared with preevisceration wash. Although EO-I alone or a sequential treatment of acidic and alkaline EO additionally reduced numbers of *E. coli* O157:H7, these effects were not different ($P < 0.05$) from each other. EO was not as effective in reducing *E. coli* O157:H7 on the surfaces of cheek meat as the other compounds tested. This may be due to organic materials like proteins, fats, and oils that could shield the bacterial cells from bactericidal activity of chlorine and low pH of EO *(24).*

For OZ treatments, OZ alone, an HP wash, or a sequential treatment of an HP wash and then a final rinsing with OZ did not ($P > 0.05$) reduce *E. coli* O157:H7 lower than those levels after preevisceration wash (Table 2). Castillo et al. *(5)* reported that OZ (95 ppm) at 80 lb/in^2 and 28°C did not significantly reduce *E. coli* O157:H7 and *Salmonella* Typhimurium on inoculated beef carcass surfaces compared with water wash at 200 to 400 lb/in^2 at 35°C. Reagan et al. *(20)* also reported that OZ treatment (0.3 to 2.3 ppm) at 20 lb/in^2 had only a minor effect and was equivalent to water wash between 28 and 42°C at 60 to 400

TABLE 2. *Effect of various interventions on bacterial contamination of bovine head inoculated with* E. coli *O157:H7*

Treatment group[a]	*n*	Log CFU/cm^2[b]	Log CFU/cm^2 reduction from main treatment[c]
Control	40	6.08 A	—
Prewash	40	4.25 B	1.83
Hot water	40	2.53 C	1.72 A
Control	40	5.84 A	—
Prewash	40	4.28 B	1.56
Lactic acid	40	2.76 C	1.52 AB
Control	40	6.27 A	—
Prewash	40	4.66 B	1.61
FreshFx	40	3.6 C	1.06 B
Control	40	6.31 A	—
Prewash	40	4.8 B	1.51
EO-I	40	4.48 C	0.32 C
Control	40	6.18 A	—
Prewash	40	5.05 B	1.13
EO-II	40	4.82 C	0.23 C
Control	40	5.56 A	—
Prewash	40	3.32 B	2.24
OZ-I	40	2.93 B	0.39 C
Control	40	5.58 A	—
Prewash	40	3.59 B	1.99
HP	40	3.41 B	0.18 C
OZ-II	40	3.37 B	0.04 C

[a] Control was not subjected to any treatment and served as initial populations of *E. coli* O157:H7. Prewash, simulated preevisceration wash of water at 25°C for 10 s at 45 lb/in^2, followed by 74°C for 10 s at 10 lb/in^2. Hot water was used at 10 lb/in^2 at 74°C. Lactic acid and FreshFx were used as final rinse at 25 lb/in^2 at 25°C. EO-I, acidic electrolyzed oxidizing water as a final rinse at 25 lb/in^2 and 25°C. EO-II, a sequential treatment of 13 s of alkaline electrolyzed water at 25 lb/in^2 and 25°C and a final rinse with 13 s of acidic electrolyzed water at 25 lb/in^2 and 25°C. OZ-I, ozonated water was used as a final rinse at 25 lb/in^2 and 25°C; HP, high-pressure wash at 145 lb/in^2 and 25°C. OZ-II, a sequential treatment of 6 s of high-pressure water wash (HP) at 145 lb/in^2 and 25°C and a final rinse with 20 s of ozonated water.

[b] Means in the column within treatment group bearing the same letter do not differ significantly at $P \leq 0.05$.

[c] Means in the same column across treatments bearing the same letter do not differ significantly at $P \leq 0.05$.

lb/in^2 in reducing *E. coli* on inoculated beef carcasses. Based on the results of this study, both EO and OZ treatments were not effective as decontamination strategies for the reduction of *E. coli* O157:H7. Hot water, lactic acid, and FreshFx could be used as antimicrobial treatments to reduce *E. coli* O157:H7 on bovine head and cheek meat.

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